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मानक

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IS 5055 (1996): Lecithin, Food Grade [FAD 8: Food Additives]



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“Knowledge is such a treasure which cannot be stolen”

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IS 5055 : 1996

भारतीय मानक
लेसिथीन, खाद्य ग्रेड — विशिष्टि
(पहला पुनरीक्षण)

Indian Standard
LECITHIN, FOOD GRADE — SPECIFICATION
(*First Revision*)

ICS 67 220 20

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BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

September 1996

Price Group 4

AMENDMENT NO. 1 FEBRUARY 2006
TO
IS 5055 : 1996 LECITHIN, FOOD GRADE —
SPECIFICATION

(First Revision)

[Page 1, Table 1, Sl No (viii), col 3] — Substitute '10' for '100'

(FAD 8)

FOREWORD

This Indian Standard (First Revision) was adopted by the Bureau of Indian Standards, after the draft finalized by the Food Additives Sectional Committee had been approved by the Food and Agriculture Division Council

With the increased production of processed foods, manufacturers have started adding a large number of substances, generally in small quantities, to improve the appearance, flavour, texture or storage properties of the processed foods. As certain impurities in these substances could be harmful, it is necessary to have a strict quality control of these food additives. A series of standards was, therefore, prepared by this Institution to cover purity and identification of these substances. These standards would help in checking purity, which requires to be checked at the stage of manufacture, for it is extremely difficult (and in many cases impossible) to detect the impurity once these substances have been added to the processed foods. Besides, these standards are intended to guide the indigenous manufacturers in making their product conform to specifications that are accepted by scientists, health authorities and international bodies, and the consumer industries to use them within the quantity permitted by the health authorities.

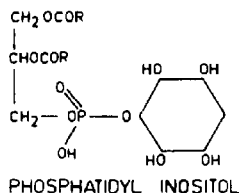
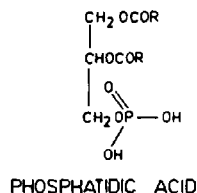
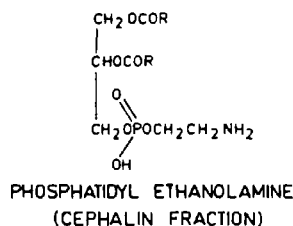
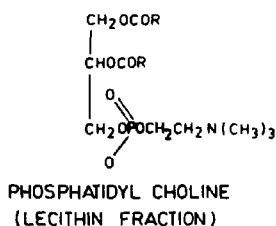
Lecithin, widely used as anti-oxidant and emulsifier, is permitted under the *Prevention of Food Adulteration Rules, 1955*, as well as the *Fruit Product Order, 1955*.

This standard was first issued in 1969. It is being revised to make the following changes/additions:

- To upgrade the standard by increasing the purity limit
- To provide a separate clause for description including the solubility property to keep it in line with food chemical codex NRC
- To incorporate the amendment No 1 issued to earlier version
- To include the requirements for heavy metals and peroxide value, and their methods of test
- To provide information as to whether it is of animal origin or vegetable origin or both and the expiry date under marking clause

Chemical Names and Formulae

The recognized chemical names are lecithin, phospholitein, phosphatides, and phospholipids. Food grade lecithin is a complex mixture of acetone insoluble phosphatides consisting chiefly of phosphatidyl choline, phosphatidyl ethanolamine, phosphatidic acid, and phosphatidyl inositol combined with various amounts of other substances, such as triglycerides, fatty acid and carbohydrates. Formulae for various phosphatides are given below:



R = various saturated and unsaturated fatty acids groups

(Continued on third cover page)

(Continued from second cover page)

In the preparation of this standard considerable amount of assistance has been derived from the Food Chemical Codex, Third Edition, National Academy of Sciences, National Research Council, Washington DC, USA

For the purpose of deciding whether a particular requirement of this standard is complied with the final value observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 - 1960 'Rules for rounding off numerical values (revised)' The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard

Indian Standard

LECITHIN, FOOD GRADE — SPECIFICATION

(First Revision)

1 SCOPE

This standard prescribes the requirements and the methods of sampling and tests for lecithin, food grade

2 REFERENCES

IS No	Title
1070 1992	Reagent grade water (<i>third revision</i>)
1699 1995	Methods of sampling and test for synthetic food colours (<i>second revision</i>)
5306 1996	Sodium carboxymethyl cellulose, food grade (<i>second revision</i>)

3 DESCRIPTION

3.1 The material is a viscous semi-liquid with a characteristic odour. It is light yellow to brown depending upon whether it is bleached or unbleached. Lecithin is obtained from egg or edible vegetable oilseeds by suitable dehydration or solvent extraction using food grade solvents. It may also be obtained from animal sources. Edible diluents, such as cocoa butter and vegetable oils may be added to improve functional and flavour characteristics.

3.2 The material is insoluble in water but characteristically hydrated with swelling. It is insoluble in acetone but soluble in chloroform and benzene. The 'lecithin fraction' is soluble while cephalin fraction is insoluble in ethanol.

NOTE: The solubility is intended only as information regarding approximate solubility and is not to be considered as a quality requirement and is of minor significance as a means of identification or determination of purity and dependence must be placed on other specifications.

4 REQUIREMENTS

4.1 Identification

4.1.1 Yellow Precipitate with Ammonium Molybdate

Ignite 1 g of the material with 2 g of anhydrous sodium carbonate. Cool and dissolve the residue in 5 ml of water and 5 ml of nitric acid. Add 5 ml of ammonium molybdate and heat to boiling. A yellow precipitate shall be formed.

4.1.2 Blue Precipitate with Ferrous Sulphate

Fuse about 0.5 g of the material with about 0.05 g of

sodium in a soft glass tube, and heat to redness. Plunge while hot into about 10 ml of distilled water, heat to boiling and filter. Add a few crystals of ferrous sulphate to the filtrate, boil and add dilute sulphuric acid until just acidic. Allow to stand for 15 minutes, filter and wash. A blue precipitate shall be formed.

4.1.3 Reflux 1 g of lecithin for 1 hour with 25 ml of 0.5 N ethanolic potassium hydroxide. When cooled to 0°C a precipitate of potassium soap shall be obtained.

4.2 Gossypol Test

The total gossypol content in cottonseed lecithin shall not exceed 5 percent by mass. The method for determination of gossypol is given in Annex A.

4.3 The material shall also conform to the requirements given in Table 1.

Table 1 Requirements for Lecithin, Food Grade

Sl No	Characteristic	Requirement	Method of Test, Ref to	
			Annex of this Standard	Clause of IS 1699 1995
(1)	(2)	(3)	(4)	(5)
i)	Purity, as acetone-insoluble residue, percent by mass, <i>Min</i>	62*	B	—
ii)	Moisture, percent by mass, <i>Max</i>	2	C	
iii)	Benzene insoluble matter, percent by mass, <i>Max</i>	0.3	F	
iv)	Acid value, <i>Max</i>	35	F	
v)	Arsenic (as As), mg/kg, <i>Max</i>	3		15
vi)	Lead (as Pb), mg/kg, <i>Max</i>	10		15
vii)	Heavy metals as (Pb) mg/kg, <i>Max</i>	40	G	
viii)	Peroxide value, <i>Max</i>	100	H	

*Equivalents to 2.2 percent of phosphorous when determined, by the method given in Annex 1)

5 PACKING AND MARKING

5.1 Packing

The material shall be securely packed in well-filled containers with minimum access to light and air. The

IS 5055 : 1996

containers shall be such as to preclude contamination of the contents with metals or other impurities

5.2 Marking

Each container shall be legibly and indelibly marked with the following information

- a) Name of the material including the words 'Food Grade',
- b) Name and address of the manufacturer,
- c) Minimum net content,
- d) Batch or code number,
- e) Whether made from vegetable origin or animal origin or both,
- f) Expiry date, and
- g) Any other requirements as specified under the *Standards of Weights and Measures (Packaged Commodities) Rules, 1977/Prevention of Food Adulteration Rules, 1955*

5.2.1 BIS Certification Marking

The product may also be marked with the Standard Mark

5.2.1.1 The use of the Standard Mark is governed by the provisions of the *Bureau of Indian Standards Act, 1986* and the Rules and Regulations made thereunder. The details of conditions under which the licence for the use of the Standard Mark may be granted to manufacturers or producers may be obtained from the Bureau of Indian Standards

6 SAMPLING

Representative samples of the material shall be drawn according to the method prescribed in 4 of IS 1699 1995

7 QUALITY OF REAGENTS

Unless specified otherwise, pure chemicals and distilled water (see IS 1070 1992) shall be employed in tests

NOTE — Pure chemicals shall mean chemicals that do not contain impurities which affect the experimental results

ANNEX A (Clause 4.2)

DETERMINATION OF TOTAL GOSSYPOL

A-1 PRINCIPLE

The term 'total gossypol' designates 'free gossypol' 'bound gossypol' and closely related pigments which after hydrolysis and reaction with an organic amine (*p*-anisidine or aniline) give a product identical spectrophotometrically with that obtained from pure gossypol and the same reagent. In this method, total gossypol is completely removed from lecithin in a 30-minute extraction during which gossypol is complexed with neutralized 3-amino-1-propanol in dimethyl formamide. The difference in absorption of aliquot portions of the extract before and after reaction with aniline serves as a measure of the total gossypol content, and allows proper correction for the background absorption of the extracts

A-2 APPARATUS

A-2.1 Photoelectric Colorimeter — With a filter having a maximum transmittance in the vicinity of 440 nm or a spectrophotometer isolating a band at 440 nm

A-2.2 Pipettes, Volumetric — 1, 2, 4, 6, 8 and 10 ml

A-2.3 Flasks, Volumetric — 25, 50, and 100 ml

A-2.4 Insulated Water Bath

Thermostatically controlled to $\pm 1^{\circ}\text{C}$, and capable of

keeping the water at a gentle boil. The water-bath should be equipped with clamps to securely hold the volumetric flasks immersed in water

A-3 REAGENTS

A-3.1 *iso*Propyl Alcohol-Hexane Mixture

Mix 60 volumes of reagent grade *iso*propyl alcohol and 40 volumes of commercial hexane

A-3.2 Complexing Reagent

Pipette 2 ml of 3-amino-1-propanol and 10 ml of glacial acetic acid into a 100-ml volumetric flask, cool to room temperature, and dilute to volume with dimethyl formamide (N-N dimethyl formamide, redistilled between 152° to 153°C). This reagent is stable for one week after preparation

NOTE — 3-amino-1-propanol may be redistilled if coloured. Its boiling point is 188°C, and it may be conveniently distilled under water pump vacuum

A-3.3 Aniline

Reagent grade, redistilled over zinc dust. Store in a refrigerator and redistil when the absorbance of the reagent blank exceeds 0.022

A-4 STANDARD GOSSYPOL SOLUTIONS

A-4 STANDARD GOSSYPOL SOLUTIONS

A-4.1 Weigh accurately 25 mg of pure gossypol or 27.9 mg of pure gossypol acetate, dissolve in and make up to 50 ml volume with the complexing reagent. If exactly 25 mg of pure gossypol is used, the solution shall contain 0.5 mg/ml.

A-4.2 Calibration Curve

Pipette 2, 4, 6, 8 and 10 ml of standard gossypol solution into 50-ml volumetric flasks.

A-4.3 To each standard add sufficient complexing reagent to make up the total volume to 10 ml. Use 10 ml of the complexing reagent as a blank.

A-4.4 Heat the flask containing the standards and the blank in a boiling water bath (100°C) for 30 minutes, cool and dilute to volume with the isopropyl alcohol-hexane mixture.

A-4.5 Pipette in duplicate 2 ml aliquots of each diluted standard and of the blank into 25-ml volumetric flasks. Dilute one set of aliquots to volume with the isopropyl alcohol-hexane mixture and reserve as *reference solutions*.

A-4.6 To the other set of aliquots, add 2 ml of aniline, heat in a boiling water-bath for 30 minutes, cool to room temperature and dilute to volume with isopropyl alcohol-hexane mixture. Allow the flask to stand at room temperature for one hour after dilution and mixing.

A-4.7 With a spectrophotometer, determine the absorbance of the reagent blank at 440 nm using the dilute blank aliquot without aniline as a reference solution.

A-4.8 Determine the absorbance of each gossypol standard reacted with aniline, using the appropriate diluted standard as a reference solution. Subtract the absorbance of the reagent blank from that of each standard to obtain the corrected absorbance.

A-4.9 Calculate the calibration factor by dividing the number of milligrams of gossypol in the 2 ml aliquot of each standard by the appropriate corrected absorbance. Average the factors for all the gossypol standards. When a photo-electric colorimeter is used, the factors

shall probably vary with each concentration of gossypol in which case a calibration curve should be plotted and used.

A-5 SAMPLE PREPARATION

Heat the sample to approximately 50°C, mix well and filter. Store at 0°C, if analysis is not done immediately. The analytical sample should contain from 1 to 5 mg of gossypol. For maximum precision, the aliquot used should contain 0.1 mg of gossypol.

A-6 PROCEDURE

A-6.1 Weigh sufficient quantity of the sample material as directed in A-5.1 into a 50-ml volumetric flask. Add 10 ml of the complexing reagent.

A-6.2 Use 10 ml of the complexing reagent as the reagent blank.

A-6.3 Heat both the sample and the blank in a boiling water-bath for 30 minutes, cool to room temperature, dilute to volume with isopropyl alcohol hexane mixture and mix. Filter through Whatman No. 1 or equivalent filter paper and collect the filtrate in a small glass stoppered flask.

A-6.4 Pipette duplicate aliquots (*see* A-5.1) of the filtered extract and of the reagent blank into 25-ml volumetric flasks.

A-6.5 Dilute one of the aliquots to volume with the isopropyl alcohol hexane mixture and reserve as *reference solutions*.

A-6.6 To the other aliquot, add 2 ml of aniline, develop the colour and determine the corrected absorbance at 440 nm as outlined in A-4.7 and A-4.8.

A-7 CALCULATION

Determine the gossypol (in milligrams) in the sample aliquot by means of the calibration curve on the calibration factor.

Total gossypol, percent by mass =
$$\frac{5 \times \text{mg gossypol in sample aliquot}}{\text{Mass of sample in g} \times \text{Volume of aliquot used for analysis}}$$

ANNEX B[Table 1, *Sl No (i)*]**DETERMINATION OF PURITY****B-1 REAGENTS****B-1.1 Purification of Phosphatides**

Dissolve 5 g of phosphatides from previous acetone insoluble matter determination in 10 ml of petroleum ether and add 25 ml of acetone to the solution. Transfer approximately equal portions of the precipitate to each of two 40-ml centrifuge tubes using additional portions of acetone to facilitate the transfer. Stir thoroughly, dilute to 40 ml with acetone, stir again, chill for 15 min in an ice-bath, stir again, and then centrifuge for 5 min. Decant the acetone, crush the solids with a stirring rod, refill the tube with acetone, stir, chill, centrifuge, and decant as before. The solids after the second centrifugation require no further purification and may be used for preparing the phosphatide acetone solution. Five grams of the purified phosphatides are required to saturate about 16 litres of acetone.

B-1.2 Phosphatide Acetone Solution

Add a quantity of purified phosphatides to sufficient acetone previously cooled to a temperature of about 5°C to form a saturated solution, and maintain the mixture at this temperature for 2 hours, shaking it vigorously at 15 minutes intervals. Decant the solution through a rapid filter paper avoiding the transfer of any undissolved solids to the paper and conducting the filtration under refrigerated conditions (not above 5°C).

B-2 PROCEDURE

Soften a portion of the material by warming it in a water bath at a temperature not exceeding 60°C and then mixing it thoroughly. Transfer 2 g of a well-mixed sample accurately weighed into a 40-ml centrifuge tube previously tared with a glass stirring rod. Add 15 ml of phosphatide acetone solution from a burette. Warm the

mixture in a water bath until the lecithin melts, but avoid evaporation of the acetone. Stir until the sample is completely disintegrated and dispersed, and then transfer the tube into an ice bath, chill for 5 minutes, remove from the ice bath, and add about one half of the required volume of phosphatide acetone solution, previously chilled for 5 minutes in an ice bath. Stir the mixture to complete dispersion of the sample, dilute to 40 ml with chilled phosphatide acetone solution (5°C), again stir and return the tube and contents to the ice bath for 15 minutes. At the end of the 15 minutes chilling period stir again while still in the ice bath, remove the stirring rod, temporarily supporting it in a vertical upside down position, and centrifuge the mixture immediately at about 2 000 rev/min for 5 minutes. Decant the supernatant liquid from the centrifuge tube, crush the centrifuged solids with the same stirring rod previously used, and refill the tube to the 40-ml mark with chilled (5°C) phosphatide acetone solution, and repeat the chilling, stirring, centrifugation, and decantation procedure previously followed. After the second centrifugation and decantation of the supernatant acetone, again crush the solids with the assigned stirring rod, and place the tube and its contents in a horizontal position at room temperature until the excess acetone has evaporated. Mix the residue again, dry the centrifuge tube and its contents at 105°C for 45 min in a forced draft oven, cool and weigh.

B-3 CALCULATION

Acetone insoluble residue
percent by mass = $(100 R/S) - B$

where

R = mass of the residue,

S = mass of the sample, and

B = benzene insoluble matter (see E-2.1)

ANNEX C[Table 1, *Sl No. (ii)*]**DETERMINATION OF MOISTURE****C-1 APPARATUS**

C-1.1 Oven — maintained at $105^\circ \pm 1^\circ\text{C}$

C-1.2 Weighing Bottle — glass stoppered, shallow

C-2 PROCEDURE

C-2.1 Weigh accurately about 10 g of the well mixed sample in the tared weighing bottle. Distribute the

sample as evenly as practicable to a depth of about 5 mm. Place the bottle containing the sample (uncovered) in the oven maintained at $105^\circ \pm 1^\circ\text{C}$. Remove the bottle from the oven after one hour, close the bottle promptly and allow it to come to room temperature in a desiccator. Weigh it.

C-2.2 Calculate loss on drying, percent by mass

ANNEX D

(Table 1)

DETERMINATION OF PHOSPHORUS

D-1 REAGENTS

D-1.1 Ammonia Solution — 2.5 percent (w/v)

D-1.2 Potassium Nitrite

D-1.3 Sodium Carbonate — anhydrous

D-1.4 Magnesium Amino Sulphate

Dissolve 10 g of magnesium sulphate and 20 g of ammonium chloride in 80 ml of water and 42 ml of dilute ammonia solution set aside for a few days in a well-closed bottle decant and filter

D-1.5 Nitric Acid — Dilute

D-2 PROCEDURE

Mix about 0.5 g, accurately-weighed sample with 5 g of a mixture of equal parts of anhydrous sodium carbonate and potassium nitrite. Place the material in a crucible cover it with a layer of anhydrous sodium carbonate and ignite. Dissolve the residue in dilute nitric acid and make it alkaline with ammonia solution and add a slight excess of solution of magnesium amino sulphate and filter. Wash the precipitate with ammonia solution (2.5 percent m/v), ignite and weigh the residue of magnesium pyrophosphate ($Mg_2P_2O_7$). One gram of the residue is equivalent to 0.2786 g of phosphorus.

ANNEX E

[Table 1, Sl No (iii)]

DETERMINATION OF BENZENE INSOLUBLE MATTER

E-1 PROCEDURE

Soften a portion of the material by warming it at a temperature not exceeding 60°C and then mix it thoroughly. Weigh 10 g of a previously well-mixed sample into a 250-ml wide-mouth Erlenmeyer flask add 100 ml of benzene and shake until the lumps are dissolved. Filter the solution through a 30 ml Corning C⁺ porosity or equivalent filtering funnel which previously has been dried at

105°C for an hour cooled in a desiccator and weighed. Wash the flask with two successive 25-ml portions of benzene and pass the washings through the filter. Dry the funnel at 105°C for an hour cool to room temperature in a desiccator, and weigh.

E-2 CALCULATION

From the gain in mass of the funnel calculate the percent of the benzene insoluble matter in the sample.

ANNEX F

[Table 1, Sl No (iv)]

DETERMINATION OF ACID VALUE

F-1 PROCEDURE

Soften a portion of the material by warming it in a water bath at a temperature not exceeding 60°C and then mix it thoroughly. Transfer about 2 g of the well-mixed sample into a 250-ml wide-mouth Erlenmeyer flask, and dissolve it in 50 ml of petroleum ether. To this solution, add 50 ml of alcohol, previously neutralized to phenolphthalein with 0.1 N sodium hydroxide, and mix well. Add phenolphthalein and titrate with 0.1 N sodium

hydroxide to a pink end point which persists for 5 seconds.

F-2 CALCULATION

Calculate the number of milligrams of potassium hydroxide required to neutralize the acids in one gram of the sample by multiplying the number of millilitres of 0.1 N sodium hydroxide consumed in the titration by 50 and dividing the result by the weight of the sample.

ANNEX G

[Table 1, *Sl No* (vii)]

DETERMINATION OF HEAVY METALS

G-1 Proceed as given in Annex F of IS 5306 : 1996 except that **F-2.3** shall be as follows

To each tube add 10 ml of freshly prepared

hydrogen sulphide, mix and allow to stand for 5 min and view over a white surface. The colour of Solution B shall not be darker than that of Solution A.

ANNEX H

[Table 1, *Sl No* (viii)]

DETERMINATION OF THE PEROXIDE VALUE

H-0 PRINCIPLE

Oxidation of potassium iodide by the peroxides of lecithin and titration of the iodine liberated using standard sodium thiosulphate solution

H-1 APPARATUS

H-1.1 Analytical Balance

H-1.2 Apparatus – As shown in Fig. 1

H-2 REAGENTS

H-2.1 Acetic Acid Glacial

H-2.2 Chloroform

H-2.3 Potassium Iodide

H-2.4 Sodium Thiosulphate (0.1 mol/l or 0.01 mol/l)

H-2.5 Starch Solution (approximately 1 percent m/v)

H-3 PROCEDURE

H-3.1 Place 10 ml of glacial acetic acid and 10 ml of chloroform in the 100-ml flask. Fit the glass tube and reflux condenser and gently boil the mixture for two minutes to expel all dissolved air. Dissolve 1 g of potassium iodide in 1.3 ml of water and add this solution to the mixture in the flask taking care that the boiling is not interrupted.

H-3.2 If a yellow colour appears at this stage the determination must be rejected and repeated using fresh reagents.

H-3.3 Accurately weigh, to the nearest mg, about 1 g of the sample and, after a further two minutes of boiling, add the weighed sample to the contents of the flask again taking care that the boiling remains continuous. For this purpose the sample should be contained in a microbeaker which may be lowered through the glass tube with a glass rod, the bottom of which has been suitably shaped as shown in the diagram. The condenser may be removed for a short time. Continue

boiling for three to four minutes. Stop heating and immediately disconnect the condenser. Quickly add 50 ml of water through the glass tube. Remove the glass tube and cool the flask to room temperature under the water tap. Titrate with sodium thiosulphate until the aqueous layer becomes pale yellow. Add 1 ml of starch solution and continue the titration until the blue colour is discharged. Shake the flask well during the titration to ensure the complete extraction of iodine from the non-aqueous layer.

H-3.4 Obtain a blank titration value by repeating the complete procedure (**H-3.1** to **H-3.3**) but without adding the sample.

H-4 CALCULATION

The Peroxide value in the sample, in milliequivalents per kilogram, is given by

$$\frac{1000 \times a \times (V_1 - V_2)}{m_0}$$

where

V_1 = volume in millilitres of thiosulphate solution required for the titration of the sample

V_2 = volume in millilitres of thiosulphate solution required for the titration of blank,

a = concentration of sodium thiosulphate solution in mol/l, and

m_0 = initial mass in grams of the sample taken

NOTES

1 The choice of the concentration of the sodium thiosulphate used depends on the anticipated titration value. If less than 0.5 ml of 0.1 mol/l sodium thiosulphate is required, repeat the determination using 0.01 mol/l sodium thiosulphate.

2 The determination should not be carried out in strong light.

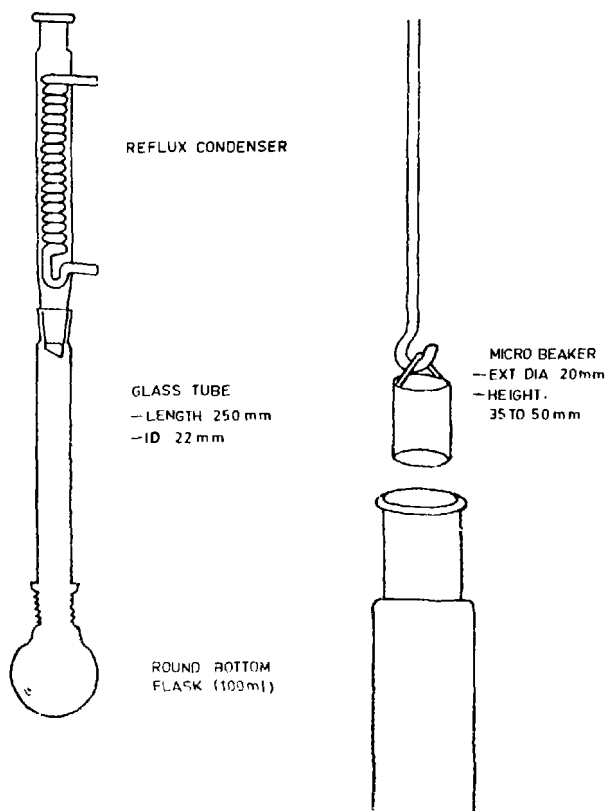


FIG 1 APPARATUS FOR THE DETERMINATION OF PEROXIDE VALUE

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Review of Indian Standards

Amendments are issued to standards as the need arises on the basis of comments. Standards are also reviewed periodically, a standard along with amendments is reaffirmed when such review indicates that no changes are needed, if the review indicates that changes are needed, it is taken up for revision. Users of Indian Standards should ascertain that they are in possession of the latest amendments or edition by referring to the latest issue of 'BIS Handbook' and 'Standards Monthly Additions'

This Indian Standard has been developed from Doc No FAD 8 (467)

Amendments Issued Since Publication

Amend No	Date of Issue	Text Affected

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